14P.

SEMI-ANNUAL STATUS REPORT

Grant Number:	NsG-170-61
Period:	1 August 1963 to 31 January 1964
Report Number:	Four (4)
Contractor:	Regents of the University of California, Berkeley
Project Title:	"Experimental Research on Hemodynamic and Metabolic Functions in Primates"
Principal Investigator:	Nello Pace, Ph.D., Professor of Physiology Department of Physiology University of California, Berkeley
Other Professional Personnel:	Norman J. Barnstein, M.D.; Mott D. Cannon, M.D., Rutherford S. Gilfillan, M.D., Benjamin W. Grunbaum, Ph.D., Arthur M. Kodama, Ph.D., and Donald F. Rahlmann, Ph.D.

Objective: To establish physiological base line data and to develop instrumentation necessary for the automatic measurement of hemodynamic and metabolic parameters on primates during prolonged periods of weightlessness.

Status: During the six months period of this report the primate colony has been maintained in general good health, as evidenced by a steady weight gain by each animal. Rigid quarantine procedures for tuberculosis and intestinal tract pathogens continue to be effective in preventing their introduction by new arrivals in the colony. However, one member of the colony had to be removed because of the appearance of malaria organisms in the blood together with typical symptoms. At the end of this report period the colony comprises 14 male and 4 female pig-tailed monkeys (Macaca nemestrina), 2 male rhesus monkeys (Macaca mulatta), one male stump-tailed monkey (Macaca speciosa) and one male squirrel monkey (Saimiri sciurea). The bulk of the physiological observations

RC#1

continue to be made on the pig-tailed monkeys, primarily because of their suitability for the projected weightlessness studies in the Biosatellite Project.

Two new general procedures, one for long-term restraint and the other for chronic vascular catheter implantation, have been developed and are in process of critical test. Suprapubic cystostomy is also being examined as a method of effecting chronic bladder catheterization for quantitative urine collection, thus far carried out successfully for as long as two weeks without adverse effect on the animal.

The restraint technique now in use is based on the fiberglass couch developed earlier under this project and which includes a plexiglas lap shield to isolate the lower and upper halves of the animal from each other. A nylon net jacket for the monkey has been designed in such a way that it acts further to prevent the animal's hands from touching his body below the waist or on the back. The jacket also has straps which fasten through slots in the couch behind the animal to hold his upper torso snugly yet comfortably against the couch. The couch itself incorporates metal rails which slide in vertical tracks attached inside a standard 55-gallon steel drum with a removable lid. Thus the couch containing the comfortably restrained monkey in a sitting position can be lowered into the drum for cheap and effective simulation of a spacecraft capsule. It also makes possible a wide variety of physiological measurements on an isolated, unanesthetized, comfortably restrained animal. In particular, the restraint couch configuration makes feasible the chronic implantation of vascular and bladder catheters which are led out through the wall of the drum, and connected to automatic instrumentation and left unattended for days at a time.

A new surgical procedure has been developed for the vascular catheter implantations. Earlier, catheters were introduced through an incision in the wall of the inferior vena cava or of the abdominal aorta after retroperitoneal exposure of the vessels following incision through the musculature of the belly wall. The catheters were tied into the vessel wall by means of a purse-string suture, and then were led out through an appropriately directed needle puncture through the dorsal body wall at about the level of the kidneys. Although this procedure has been quite successful in terms of maintaining asepsis and catheter patency for periods up to six months, occasional loss of a preparation has occurred through development and rupture of an aneurysm about the entrance site of the aortic catheter. Accordingly, a more positive procedure has been utilized for sealing in the catheter. In the new procedure thoracotomy is performed and the catheter is inserted into the left subclavian artery about one centimeter from the aorta and passed retrograde toward the heart until the tip of the catheter is just inside the aorta. The catheter is tied in securely by suture loops passed around the subclavian artery. The artery is also tied off peripheral to the point of entrance of the catheter. In a separate operation a week apart the superior vena cava is catheterized in analogous fashion by way of the azygous vein; in this case the tip of the catheter is brought to lie very near the entrance to the right atrium. Both the arterial and the venous catheters are brought out of the thoracic cage through the dorsal portion of the intercostal incision.

Analytical chemical procedures for the following urinary constituents are presently in operation: total osmotic activity, sodium, potassium, calcium, magnesium, ammonia, chloride, phosphate, sulfate, urea, uric acid, creatinine, glucose, 5-hydroxyindoleacetic acid, 17-ketosteroids,

17-hydroxycorticosteroids, epinephrine, norepinephrine and dopamine. All of these can be done on urine which has been preserved by freezing. In addition, urine pH, bicarbonate and titratable acidity can be measured on fresh urine. An automatic urine collector has been developed to the point where all but the freezing of the collected specimens has been accomplished successfully. As soon as this last feature is realized routine analysis of pig-tailed monkey urine will begin.

A procedure for in vivo measurement of total body water content by means of tritium-labeled water is now operational in the laboratory. A new in vivo method, based on Boyle's Law, for determination of total body volume is currently under development. If successful, this will make possible computation of body density, and hence estimation of total body fat content. It is planned to use these techniques to measure body composition of the pig-tailed monkey.

The new physiological base line data obtained on the pig-tailed monkey during the six months period of the report are summarized in the following sections.

Reproduction and Growth

On 17 December 1963, following a gestation period of approximately 170 days, a female pig-tailed monkey from our colony gave birth, without complications, to a single male offspring. In estimating the gestation period, the day of conception was assumed to be the same as the day of ovulation, which in turn was assumed to be the fifteenth day following the onset of the last menses. Prior to exposure to a fertile male, the female had exhibited two menstrual cycles of 28 days and 30 days. Day one of the cycle was considered to be the first day of menses.

Regular X-ray and anthropoidimetric measurements of this male of known birth date will permit precise description of skeletal growth

changes in relation to chronological age. Similar data are being collected on a female pig-tailed monkey of known birth date. Changes in linear measurement of various parts of the body of this female from 6 months to 2 years of age are presented in Table 1.

Motile sperm cells from the caudal epididymis were collected with a needle and syringe from two anesthetized pig-tailed monkeys. From fixed stained smears, morphological measurements of the head and tail were obtained. Mean head length was found to be 6.6 μ, and mean tail length 58.8 μ. Detailed results are shown in Table 2. By comparison, values for ejaculated human spermatozoa are approximately 5 μ for the head length and 45 μ for the length of the tail.

Hematology

Blood samples were collected from unanesthetized monkeys by venipuncture of the lesser saphenous vein. Additional venous and arterial blood samples were obtained from indwelling catheters. Hematological data from 8 male and 3 female monkeys are presented in Table 3.

Hemodynamics

Data on heart rate, respiratory rate and blood pressure for unanesthetized and anesthetized pig-tailed monkeys are presented in Table 4. Variations in the values obtained from the anesthetized monkeys were probably due in part to the type of anesthetic agent used and the depth of anesthesia. The time span of each trial varied from 2 hours to 30 hours. With the exception of monkey #27 all of the unanesthetized animals were removed from their cages and placed in chairs for one hour prior to initial measurements. During the trials the animals were aware of normal noise and movement within the laboratory area. Monkey #27 was measured in a restraint couch inside a steel drum as described earlier.

In this environment, he remained relatively free from outside stimuli. In animal #13 the tip of the venous catheter was actually placed in the pulmonary artery, and in animal #24 the tip was just within the right ventricle. In the other four monkeys, #20, #27, #33 and #34, the catheter tip was in the vena cava at approximately the level of the opening into the right atrium. Although negative pressures were generally seen in these four animals, positive venous pressures were noted during vocalization, drinking and other body movements.

Tables 5 and 6 summarize the hemodynamic data obtained from two unanesthetized pig-tailed monkeys with chronically implanted aortic and vena caval catheters. The single injection indicator-dilution technique was used for cardiac output determination by means of indocyanine green dye. Pulmonary circulation time was measured from the time of dye injection to the initial appearance of dye in the arterial blood. Complete circulation time was determined from the initial appearance of dye in the arterial blood to initial appearance of recirculation. An estimate of blood volume was obtained by extrapolation of the arterial dye concentration curve back to zero time, after mixing had occurred.

Systemic (peripheral) resistance was computed as the difference between mean systemic arterial pressure and mean right atrial pressure divided by cardiac output, as follows:

$$R_s = \frac{1333^{*}\left[\overline{P}_{aorta}(\text{mm Hg}) - \overline{P}_{\text{right atrium}}(\text{mm Hg})\right]}{\text{C.O.(liters/min)} \times 1000\ \text{cm}^3/\text{liter} \times 0.0167\ \text{min/sec}}$$

$$R_s = \frac{80\left[\overline{P}_{aorta}(\text{mm Hg}) - \overline{P}_{\text{right atrium}}(\text{mm Hg})\right]}{\text{C.O.(liters/min)}}\ \text{dyne sec/cm}^5$$

* $\text{dynes/cm}^2/\text{mm Hg}$

Cardiac work, actually left ventricular power, was computed as the product of mean systemic arterial pressure and cardiac output, as follows:

$$C.W. = 1333 \times \bar{P}_{aorta}(\text{mm Hg}) \times C.O.(\text{liters/min}) \times 1000 \times 0.0167$$

$$C.W. = 2.22 \times 10^{-3} \left[\bar{P}_{aorta}(\text{mm Hg}) \times C.O.(\text{liters/min})\right] \text{ watts or } 10^7 \text{ ergs/sec}$$

Body Temperature.

Rectal temperature of a pig-tailed monkey in comfortable couch restraint was measured for several days by means of a thermistor inserted 10 cm into the colon. The hourly temperature readings are shown in Table 7. The highest temperatures were recorded in the late afternoon and evening, while lowest temperatures occurred during the morning. Ambient temperature was maintained at 24°C $\pm$ 2°C. The length of the light period varied slightly from day to day, but in general was about 12 hours followed by 12 hours of darkness.

Table 1. Anthropoidimetric Measurements of a Growing Female Pig-tailed Monkey (Moth, #6, Birth Date 29 December 1961)

Measurement	Age in Months 6	12	18	24
Crown to base of tail	27.9	38.0	40.0	44.0
Crown to tip of tail	40.6	53.0	56.0	62.0
Midline around shoulder to tip of finger	30.5	33.8	35.8	41.0
Midline around hip to tip of toe	33.7	37.3	39.7	45.0
Greater trochanter to knee joint	9.2	10.8	11.9	13.0
Knee joint to lateral malleolus	8.1	11.4	12.2	13.0
Humeral head to surface radial epicondyle	7.3	9.1	10.6	11.5
Tip of olecranon to ulnar styloid	9.0	10.1	11.5	12.5
Circumference of head occipital to supraoptic (above ears)	23.0	24.4	25.0	26.0
Circumference of chest at nipples	20.1	22.9	23.8	26.0
Circumference of abdomen at umbilicus	19.5	20.8	19.8	22.0
Body Weight (kg)	1.40	1.79	2.35	2.89

Table 2. Measurement of Epididymal Sperm from Two Pig-tailed Monkeys

	Animal #5	Animal #14
Head Length (μ)	6.6 (5.9-7.4)*	6.5 (5.9-6.9)
Head Width at		
Widest portion of anterior cap (μ)	4.7 (3.9-5.3)	3.8 (3.4-5.3)
Distal perimeter of cap (μ)	3.5 (3.2-3.9)	3.3 (2.8-4.3)
Base of head (μ)	2.4 (1.6-2.8)	2.0 (1.6-2.3)
Length of Mid Piece (μ)	10.6 (9.4-11.0)	11.1 (9.7-13.3)
Length of Tail (μ)	58.1 (52.4-61.9)	59.4 (55.7-62.8)

* Numbers in parentheses are ranges.

Table 3. Hematological Data from 8 Male and 3 Female Pig-tailed Monkeys

Animal No.	#2	#3	#12	#13	#17	#24	#29	#33	#6	#30	#31	Mean		
Sex	M	M	M	M	M	M	M	M	F	F	F	M+F	M	F
Measured Values														
Weight (kg)	11.5	10.3	8.7	11.2	5.4	6.6	4.6	8.0	2.5	4.9	5.7	7.2	8.3	4.4
Hematocrit (%)	46.5	39.2	49.5	38.6	42.5	46.7	38.0	43.6	41.0	41.9	38.2	42.3	43.0	40.4
Red Blood Cells x $10^6/mm^3$	6.35	5.54	5.97	5.90	6.07	5.74	5.56	5.08	5.70	6.06	5.74	5.79	5.77	5.83
White Blood Cells x $10^3/mm^3$	8.2	9.6	20.7	16.7	10.8	14.4	9.1	17.8	6.9	18.0	14.8	13.4	13.4	13.2
Lymphocytes (%)	50.5	50.1	37.0	48.0	73.0	50.7	47.0	36.7	58.7	61.0	54.1	51.6	49.1	57.9
Monocytes (%)	5.8	9.4	8.0	5.6	1.0	2.3	2.5	2.0	0.9	0.0	0.5	3.4	4.6	0.5
Neutrophils (%)	41.0	41.5	51.9	45.0	24.0	45.1	50.5	60.3	37.3	36.5	37.6	42.8	44.9	37.3
Eosinophils (%)	1.7	2.4	3.1	1.0	2.0	1.6	0.0	0.8	2.8	2.3	2.7	1.8	1.6	2.6
Basophils (%)	1.0	0.6	1.0	0.4	0.0	0.3	0.0	0.2	0.3	0.2	0.1	0.4	0.5	0.1
Hemoglobin g/100 ml whole blood	13.8	9.8	14.2	11.7	12.3	11.5	10.9	10.1	10.3	11.8	9.8	11.5	11.8	10.6
Total Plasma Prot. g/100 ml plasma	7.1	7.2	7.2	7.6	7.4	8.4	5.8	5.8	6.1	5.3	5.9	6.7	7.1	5.8
Red Blood Cell Diameter (μ)	7.51	7.61	-	7.60	7.72	7.71	7.50	-	7.64	7.34	7.60	7.58	7.61	7.53
Computed Values														
Mean Corpuscular Volume (cu μ)	73.2	70.8	82.9	65.4	70.0	81.4	68.3	85.8	71.9	69.1	66.5	73.2	74.7	69.2
Mean Corpuscular Hemoglobin (μμg)	21.6	17.7	23.8	19.8	20.3	20.0	19.6	19.9	18.1	19.4	17.1	19.8	20.3	18.2
Mean Corpuscular Hemoglobin Conc.%	29.6	25.0	28.7	30.3	28.9	24.6	28.7	23.2	25.1	28.2	25.6	27.1	27.4	26.3
Red Blood Cell - Mean Thickness (μ)	1.67	1.57	-	1.45	1.49	1.74	1.55	-	1.57	1.63	1.47	1.57	1.58	1.56

Table 4. Heart Rate, Respiratory Rate and Blood Pressure Data from Unanesthetized and Anesthetized Pig-tailed Monkeys

	Unanesthetized							Anesthetized				
Animal No.	#13	#20	#24	#27	#33	#34	Mean	#6	#20	#24	#33	Mean
No. of trials in average	2	6	5	2	1	5		1	1	2	1	
Weight (kg)	9.8	9.5	6.9	9.4	8.2	8.0		2.62	9.5	6.9	8.2	
Sex	M	M	M	M	M	M		F	M	M	M	
Heart Rate (beats/min)	201	224	205	157	224	195	201	123	153	118	168	140
Range	174-246	180-258	174-226	138-163	220-231	160-231		-	156-190	114-159	162-174	
Respiratory Rate (breaths/min)	40	39	25	23	38	29	32	-	30	26	-	28
Range	37-45	32-48	18-30	18-26	36-42	26-36		-	-	-	-	
Aorta Systolic Press. (mm Hg)	138	159	133	120	168	125	141	-	120	111	-	116
Range	117-152	116-203	117-145	105-136	160-171	110-163		-	102-147	88-136	-	
Aorta Diastolic Press.(mm Hg)	96	109	82	80	109	73	92	-	80	70	-	75
Range	87-109	92-158	70-92	62-94	105-111	70-96		-	67-107	66-97	-	
Aorta Pulse Press. (mm Hg)	42	50	51	40	62	52	50	-	40	41	-	41
Range	30-43	24-58	35-57	35-43	45-67	40-67		-	35-40	22-49	-	
Aorta Mean Press. (mm Hg)	112	127	105	100	134	91	112	-	-	-	-	-
Range	109-114	115-137	100-124	83-108	132-137	89-102		-	86-128	81-117	-	
Venous Press. (mm Hg)	-	-2	-3	-3	-	-		-	-	-	-	-
Range	-	-7.5 to +7.0	-1 to -6	-3 to +12.5	-7 to +50	-7 to +32		-	-	-	-	
Pulmonary Artery Mean Press. (mm Hg)	7	-	-	-	-	-		-	-	-	-	
Range	6-9	-	-	-	-	-		-	-	-	-	
Right Ventricle Press.												
Systole (mm Hg)	-	-	17	-	-	-		-	-	-	-	-
Diastole (mm Hg)	-	-	0	-	-	-		-	-	-	-	-

Table 5. Hemodynamic Data during a 71 Minute Period from an Unanesthetized, Male Pig-tailed Monkey Sitting Quietly (Snug, #24, Body Weight 6.9 kg, Body Surface Area 0.43 m^2*).

Number of Measurement	1	2	3	4	5	6	Mean
Time of Day	1540	1556	1603	1631	1640	1651	
Respiratory Rate (breaths/min)	28	--	--	26	29	30	28
Heart Rate	211	213	207	207	213	219	212
Aorta Systolic Pressure (mm Hg)	137	137	141	130	138	142	138
Aorta Diastolic Pressure (mm Hg)	92	87	92	91	89	90	90
Aorta Pulse Pressure (mm Hg)	45	50	49	39	49	52	48
Aorta Mean Pressure (mm Hg)	110	108	112	104	111	110	109
Pulmonary Circulation Time (sec)	3.5	3.7	3.6	3.7	3.9	3.8	3.7
Complete Circulation Time (sec)	6.2	6.3	6.0	5.7	6.1	5.5	6.0
Beats/Complete Circulation	20.5	20.1	20.8	21.8	21.0	23.8	21.3
Blood Volume (ml)	425	428	440	431	--	443	433
Cardiac Output (liters/min)	0.70	0.66	0.71	0.67	0.63	0.67	0.67
Cardiac Index (liters/m^2/min)	1.64	1.54	1.67	1.56	1.48	1.57	1.58
Systemic Resistance (dyne sec/cm^5)	12,500	13,100	12,600	12,500	14,000	13,100	13,000
Cardiac Work (watts)	0.171	0.158	0.176	0.155	0.155	0.164	0.163
Stroke Volume (ml/beat)	3.3	3.1	3.4	3.2	3.0	3.1	3.2
Stroke Index (ml/beat/kg body wt)	0.48	0.45	0.49	0.46	0.44	0.45	0.46

* Body Surface Area in m^2 = 0.118 (Body Weight in kg)$^{2/3}$

Table 6. Hemodynamic Data during a 155 Minute Period from an Unanesthetized, Male Pig-tailed Monkey Sitting Quietly (Bardolph, #27, Body Weight 9.4 kg, Body Surface Area 0.53 m^2*).

Number of Measurement	1	2	3	4	5	6	7	8	9	10	Mean
Time of Day	1457	1510	1526	1541	1554	1610	1635	1658	1716	1732	
Respiratory Rate (breaths/min)	--	18	22	20	18	21	18	18	24	22	20
Heart Rate	156	156	144	138	156	162	180	162	186	144	158
Aorta Systolic Pressure (mm Hg)	116	119	104	110	105	108	116	130	136	128	117
Aorta Diastolic Pressure (mm Hg)	74	75	63	65	62	69	73	81	84	82	73
Aorta Pulse Pressure (mm Hg)	42	44	41	45	43	39	43	49	52	46	44
Aorta Mean Pressure (mm Hg)	95	96	83	87	83	89	95	105	110	102	94
Pulmonary Circulation Time (sec)	--	3.8	3.9	3.2	3.0	3.8	3.3	4.2	3.1	4.3	3.6
Complete Circulation Time (sec)	--	7.0	8.1	7.7	8.8	7.1	7.3	7.2	6.9	8.0	7.6
Beats/Complete Circulation	--	18.2	19.5	17.6	22.8	19.0	21.9	19.5	21.2	16.4	19.5
Blood Volume (ml)	--	--	653	--	--	--	621	615	645	615	630
Cardiac Output (liters/min)	--	0.55	0.49	0.51	0.50	0.53	0.52	0.53	0.60	0.44	0.52
Cardiac Index (liters/m^2/min)	--	1.03	0.93	0.97	0.95	1.01	0.99	1.00	1.14	0.84	0.98
Systemic Resistance (dyne sec/cm^5)	--	14,000	13,700	13,600	13,300	13,300	14,500	15,900	14,600	18,400	14,590
Cardiac Work (watts)	--	0.117	0.090	0.099	0.092	0.105	0.110	0.124	0.147	0.100	0.109
Stroke Volume (ml/beat)	--	3.5	3.4	3.7	3.2	3.3	2.9	3.3	3.3	3.1	3.3
Stroke Index (ml/beat/kg body wt)	--	0.37	0.36	0.39	0.34	0.35	0.31	0.35	0.35	0.33	0.35

* Body Surface Area in m^2 = 0.118 (Body Weight in kg)$^{2/3}$

Table 7. Rectal Temperature of a Pig-Tailed Monkey (Bardolph, #27) during a 10-Day Stay in Comfortable Couch Restraint.

	Day in Couch							
Time	3rd	4th	5th	6th	7th	8th	9th	10th
0100		39.3	39.1	38.3	-	-	38.9	39.5
0200		39.2	39.0	38.2	-	-	38.8	39.4
0300		39.2	39.0	38.4	-	-	38.7	39.2
0400		38.9	38.9	38.4	-	-	38.7	39.2
0500		38.9	38.8	38.3	-	-	38.7	39.1
0600		38.8	38.6	38.3	-	-	38.6	39.1
0700		38.8	38.5	38.3	-	38.4	38.4	39.0
0800		38.8	38.4	38.3	38.6	38.2	38.5	39.0
0900		38.8	38.4	38.2	38.7	38.4	38.7	38.9
1000		38.9	38.3	38.2	39.0	38.4	39.1	38.9
1100		39.0	38.3	38.3	-	38.5	39.2	39.0
1200		39.1	38.2	38.3	39.0	-	39.1	39.3
1300		39.2	38.4	38.2	39.0	-	39.1	39.3
1400		39.2	38.5	38.2	39.4	39.1	39.1	39.3
1500		39.3	38.5	38.4	39.6	-	39.4	39.4
1600	39.9	39.2	38.6	-	39.6	-	39.6	39.4
1700	39.7	39.4	38.6	-	40.4	39.5	39.7	39.6
1800	39.7	39.5	38.6	-	-	39.4	39.7	39.6
1900	39.6	39.5	38.5	-	39.6	39.4	39.7	
2000	39.4	39.5	38.5	-	-	39.3	39.6	
2100	39.2	39.5	38.4	-	-	39.2	39.6	
2200	39.2	39.4	38.3	-	-	39.0	39.5	
2300	39.2	39.3	38.4	-	-	39.1	39.6	
2400	39.4	39.2	38.3	-	-	39.0	39.6	